INTRODUCTION
Precise and accurate delivery of patient sample is a critical step in obtaining accurate test results. Automated analyzers have improved precision over the years, but there is still opportunity to improve further. Additionally, there is a desire to conserve sample collected from patients, leading to a need for even smaller sample volume delivery. This team set out to develop motion profiles that would achieve very high precision (<1% CV) for small volume delivery and maintain high throughout while eliminating sample carryover by employing disposable pipette tips.

BACKGROUND
Building a high throughout immunoassay analyzer that is very fast and without sample carryover is a challenge. One solution is to employ disposable pipette tips (dispo-tips). Avoiding sample carryover is important for all immunoassays.

Problem Statement
The UniCel DxI 800 was introduced in 2003 as the highest throughput immunoassay analyzer. Over the years the focus has shifted from speed to continuous improvement of assay performance. One element of assay performance is sample delivery precision. The current specification of CV <5% for 10 µL delivery is adequate, but the capability of using smaller delivery volumes is desired for future development.

Goals
The team’s aim was to focus on four key performance areas with a new pipetting subsystem:

1. Fast Cycle time (throughput > UniCel DxI 800)
2. Accurate, small sample delivery volumes (<2 µL)
3. Sample delivery precision of <5% CV at 2-µL delivery
4. Absence of sample carryover (dispo-tips)

ACRONYMS & DEFINITIONS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>SV</td>
<td>Sample vessel</td>
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<tr>
<td>DV</td>
<td>Dilution vessel</td>
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<tr>
<td>RV</td>
<td>Reaction vessel</td>
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<tr>
<td>DPP-800</td>
<td>Beckman Coulter K.K., Mishima, Japan</td>
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<tr>
<td>DPP-850</td>
<td>Beckman Coulter Inc., Chaska, MN, USA</td>
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PROTOCOL & MATERIALS

Test method
A colorimetric method for determining precision was employed. An orange-colored dye (OG) solution containing a known volume of 7% bovine serum albumin solution was introduced to the system via a sample cup. The new pipettor was programmed to deliver varying volumes into a reaction vessel (RV). Concentration of the dye was calculated, and measurements of the delivery were performed using a spectrophotometer. Three different motion profiles were created for varying ranges of volume delivery (250 µL, 25-100 µL, and 2-24 µL target). All tests, with 6 different target values at both high and low viscosity, were conducted with 10 replicates per sample, using an 8 second pipetting cycle, on 5 different pipetting subsystems.

Colorimetric method
Dye ingredient : Orange G (C16H11N2O4·2Ca·7H2O)

Instrument
Spectrophotometer : Hitachi U-3000H
Auto-dispenser : Hamilton microLAB M5615-D5
Prototype for Sample Pipetting : Did with AU pump for Hexagon pipetting

Protocol
Program required to dispense 2, 5, 10, 25, 50, 100 or 250 µL of OG into RV (0.5 mL to 125 µL, 0.25 mL to 250 µL)
Fill WB to get total 500 µL for Spect measurement
Run 10 Reps, 3 Runs for each OG volume condition

RESULT (DILUTION)

The experimental subsystem with dispo-tip + pipettor mixing successfully improved sample delivery precision across dispensing range.

RESULT (NON-DILUTION)

The experimental subsystem with dispo-tip + pipettor mixing successfully improved sample delivery precision across dispensing range.

CONCLUSION

The experimental subsystem has demonstrated performance with dispo-tip as listed below:

- CV <1.5% for 2-µL
- CV <1.0% for 5-250 µL
- 1.0-8.0 µL per sample
- 10-200x dilution rate

There are several key elements to achieve high performance for the pipetting system using the dispo-tips:

- Miniaturized Components to move together over multiple locations
- Pipettor mixing to wash out residual inside dispo-tip
- Number of pipettor mixing to mix different reagents

Having these key functionalities, the experimental subsystem would support improved assay performance when employed on an immunoassay analyzer.